Synthesis and conformational analysis of phosphorylated β-(1→2) linked mannosides

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Phosphorylated β-(1→2)-oligomannosides are found on the cell surface of several *Candida* species, including *C. albicans* (an opportunistic pathogen). These molecules are believed to take part in the invasion process of fungal infections, which in the case of *C. albicans* can lead to severe bloodstream infections and death, and can therefore be considered important from a biological standpoint. Understanding the mechanism of their action, requires access to the corresponding oligosaccharide model compounds in pure form. In the present work, synthesis of the model core structures involved in the invasion process of *C. albicans*, consisting of phosphorylated β-(1→2)-linked mannotriose and tetraose, are reported. In order to elucidate the nature of these molecules in more detail, an extensive NMR-spectroscopic study encompassing complete spectral characterization, conformational analysis and molecular modelling was performed. The obtained results were also compared to similar chemical entities devoid of the charged phosphate group.

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Introduction

Candida is a genus of fungi that causes almost 96 % of all opportunistic fungal infections. It is the fourth most commonly found pathogen in North American hospitals and the seventh in European hospitals.[1a] *Candida albicans* is the most common fungus and is part of the human microbial flora. As a result, the majority of fungal infections are caused by endogenic *C. albicans*.[1b] In usual cases, this does not cause a serious threat, but in people with compromised immune systems, such as HIV patients and people undergoing prolonged chemotherapy or treatment with antibiotics, the fungi can grow uncontrollably and cause candidiasis leading to more serious infections.[2a,b]

The cell wall of *C. albicans* contains a glycoprotein, phosphopeptidomannan, in which a backbone of α-D-(1→6) mannopyranan with various mannoside side chains is coupled to asparagine through nitrogen. The side chains consist mainly of α-(1→2)- and α-(1→3)-linked mannoses, as well as of additional α-(1→6)-linked mannose units. In addition, the cell wall contains a smaller amount of β-(1→2)-linked mannans bound to the branches either through a glycosidic bond (acid stable β-mannan) or through a phosphodiester bond (acid labile β-mannan) (Figure 1).[3]

The β-linked mannosides isolated from the phosphopeptodonannan have on multiple occasions been shown to stimulate the production of antibodies against candidasis. Consequently, similar compounds have become attractive target molecules for development of conjugate vaccines.[2a,c-e]

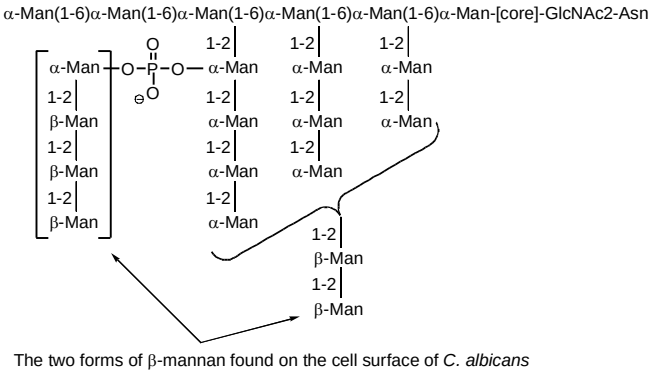


Figure 1. Portion of the *C. albicans* cell wall, containing β-(1→2)-mannosides coupled to the backbone consisting of α-D-mannopyranan.

Due to the biological properties of these molecules, they have often been subjected to extensive biological studies, including *in vitro* antibody binding studies, as well as *in vivo* studies in rabbits, mice and rats.[2,4] Extracting the molecules from living organisms is tedious, and does not usually yield sufficient amounts of material for comprehensive biological studies. This is especially true for β-linked mannosides, as they occur rather rarely, compared to some other common sugars that can be extracted and purified from nature in large scale. Only few species produce the β-linked mannosides at all, and even if they do, these molecules only make up a small portion of the total carbohydrates in these organisms. By further considering the problems associated with heterogeneity of the associated cell wall structures and the difficulties in isolating pure compounds devoid of biological contaminations, it often becomes preferable or even mandatory to turn into synthetic organic chemistry for preparative solutions.

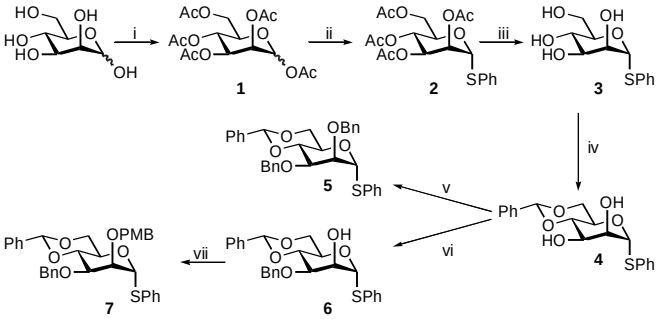
In recent years, the synthesis of β-linked mannosides has received considerable attention and reliable methodologies for their synthesis have been developed.[5] Crich and coworkers have developed methods for direct glycosylation to afford a number of β-linkages using mannopyranosyl sulphoxides and thioglycoside donors.[6] Their method is based on activation of either mannopyranosyl sulphoxides or thioglycosides with Tf_2O in the presence of a hindered base 2,4,6-tri-*tert*-butyl pyrimidine to afford an in-situ α-mannopyranosyl triflate.[7] This triflate then undergoes an S_N2-like reaction with an acceptor molecule to form a β-mannopyranoside.[8,9]

In the present work, we have focused on the synthesis of β-(1→2)-linked mannotriose and tetraose containing an anomeric α-linked phosphate group. Similar structures are found in nature on the cell surface of *C. albicans* and have been suggested to take part in the invasion process of this fungus. The final products were subjected to a detailed NMR spectroscopic and molecular modelling study in order to enhance the understanding of their conformation and nature in water solutions.

Results and Discussion

In the synthesis of oligosaccharides, one of the most challenging linkages is the β-linkage from mannose. Generally, the *trans*-1,2-glycosylation is difficult with both the kinetic and thermodynamic factors prefering the formation of the corresponding α-product. Due to the anomeric effect, electronegative substituents prefer to attain axial configurations at the anomeric position. This is due to stabilisation, as the free electrons on the ring oxygen can shift towards the anti-bonding orbital of the C-O bond (in the case of *O*-sugars). In addition, the β-face of mannose is sterically hindered due to the axial substituent on the C-2 carbon.[10] The recently developed, highly selective methodologies of Crich and coworkers have, however, made these oligosaccharides readily accessible by synthetic methods.[6-9]

Preparation of the monosaccharide building blocks utilized in the present work has been addressed previously, and their syntheses will not be discussed here in detail.[11,12] First, D-mannose was acetylated using acetic anhydride in pyridine and the resulting peracetylated mannose utilized in a subsequent $BF_3{\cdot}OEt_2$ promoted glycosylation with thiophenol to give the thioglycoside **2** in excellent yield (Scheme 1). Next, compound **2** was deacetylated under Zemplén conditions to afford **3** from which the 4,6-*O*-benzylidene protected monosaccharide **4** was prepared by reaction with $C_6H_5CH(OCH_3)_2$ in the presence of a catalytic amount of *p*-TSA. Deprotonation of **4** with NaH followed by addition of BnBr afforded the building block **5**. For synthesis of building blocks **6** and **7**, the 3-OH group of **4** was first selectively protected by forming a dibutyl stannylene between the 3-OH and 2-OH groups using Bu_2SnO, which then could be selectively benzylated using a combination of Bu_4NBr, CsF and BnBr to afford the 3-OBn derivative **6**. Finally, for synthesis of building block **7**, **6** was treated with NaH followed by addition of 4-methoxybenzyl chloride (Scheme 1).

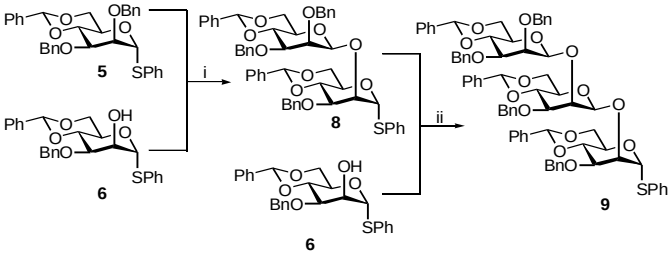


Scheme 1. Synthesis of the monosaccharide building blocks. (i) $(CH_3CO)_2O$, pyridine, r.t., 2 h (95 %); (ii) $BF_3{\cdot}OEt_2$, PhSH, CH_2Cl_2, r.t., 20 h (90 %); (iii), NaOMe, MeOH, r.t., 18 h (quant); (iv) $C_6H_5CH(OCH_3)_2$, *p*-TSA, DMF, 60 °C, 200 mbar, 2 h (91 %); (v) 1. NaH, DMF, 0 °C 0,5 h, 2. BnBr, r.t., 3 h (95 %): (vi) 1. Bu_2SnO, toluene, 120 °C reflux, 2 h, 2. Bu_4NBr, CsF, BnBr, 120 °C reflux, 3 h (85 %); (vii) 1. NaH, DMF, 0 °C, 0,5 h, 2. 4-methoxybenzyl chloride, r.t., 1 h (95 %)

Synthesis of phosphorylated mannotriose and mannotetraose

The β-coupling from mannose was achieved by the previously described methods of Crich by first activating the donors with Tf_2O in the presence of BSP and the hindered base TTBP at –60 °C to yield an in-situ α-triflate, which then was further reacted with an acceptor molecule at –78 °C. [7]

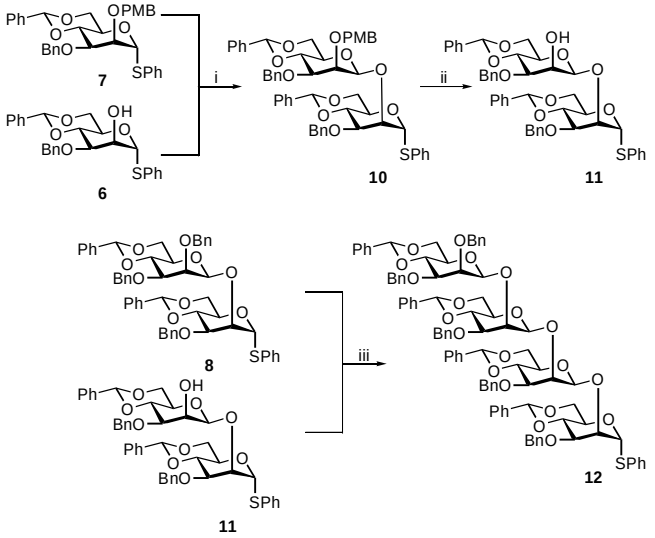
For preparation of the mannotriose, the disaccharide **8** was first prepared from monosaccharides **5** and **6** according to the earlier described method.[11] The obtained disaccharide was then coupled to monosaccharide **6**, again under similar conditions, affording the protected trisaccharide **9** in 62% yield (Scheme 2).



Scheme 2. Synthesis of the trisaccharide **9**: (i) TTBP, BSP, Tf_2O, CH_2Cl_2, –60 °C, 0,5 h → –78 °C, 2h (75 %); (ii) TTBP, BSP, Tf_2O, CH_2Cl_2, –60 °C → –78 °C, 2h (62 %).

For preparation of β-(1→2)-linked mannotetraoses, both linear 3 + 1 and convergent 2 + 2 synthetic routes could be considered. It has been shown earlier, that a 2 + 2 addition in general gives better yields and selectivities compared with the linear route.[13] This could be assigned to the helical character of β-(1→2)-linked oligomannosides, evident already at the trisaccharide stage, with the tetrasaccharide clearly adopting a helix-like structure. This prompted us to utilize the 2 + 2 addition route instead of 3 + 1, for which the disaccharide **11** had to be synthesized. The synthesis of **11** was achieved by coupling of the monosaccharides **6** and **7** to afford the protected disaccharide **10**, from which **11** could be prepared by oxidative cleavage of the 4-methoxybenzyl group using DDQ in CH_2Cl_2/H_2O. The resulting protected disaccharide **11** was then coupled with disaccharide **8** affording the protected tetrasaccharide **12** in 31 % overall yield over two steps (Scheme 3).

In general, several methodologies can be utilized to introduce phosphate groups to this type of compounds. The phosphorus bearing moiety can be added either as a nucleophile or an electrophile, depending on the functional groups incorporated. In the present study, a preferential route would involve protection of the phosphate group with protecting groups similar to those used for the carbohydrate coupling partners, allowing the final removal of all protecting groups in a single deprotection step.



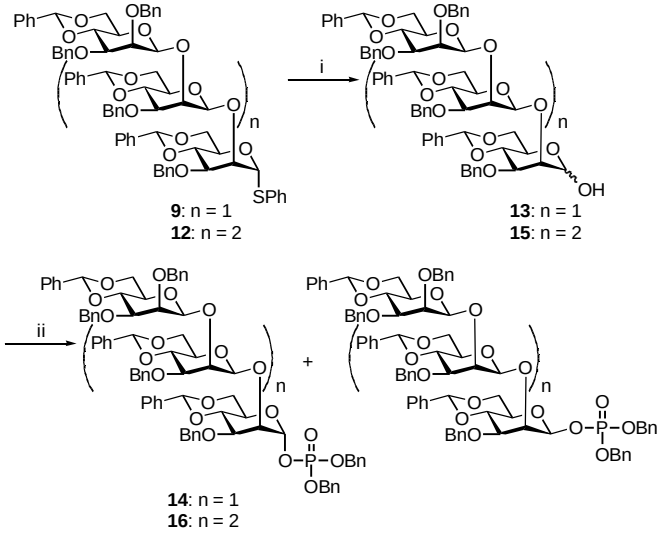
Scheme 3. Synthesis of the tetrasaccharide **12**: (i) TTBP, BSP, Tf_2O, CH_2Cl_2, –60 °C, 0,5 h → –78 °C, 2h (57 %); (ii) DDQ, CH_2Cl_2/H_2O, 0 °C, 1 h (88 %); (iii) TTBP, BSP, Tf_2O, CH_2Cl_2, –60 °C, 0,5 h → –78 °C, 2h (35 %).

Phosphorylation of similar molecules has been earlier carried out by hydrolysis of the anomeric thiophenyl group with NBS in a mixture of acetone and H_2O.[11] After the hydrolysis, the free hydroxyl group at the anomeric position can be reacted with dibenzyl(*N*,*N*-diisopropyl) phosphoramidite in the presence of 1H-tetrazole, followed by oxidation to phosphate by *m*-CPBA (Scheme 4). Unfortunately the yields from this hydrolysis were very low, possibly due to the formation of HBr, which might hydrolyse the 4,6-*O*-benzylidene protecting groups. Due to the low yields obtained with this methodology, an alternative approach was sought. Seeberger has earlier published a methodology for phosphorylation by using a thioglycoside donor and a phosphate acceptor.[14] With the protected trisaccharide **9** and tetrasaccharide **12** already containing a thioglycoside functionality, this seemed like a reasonable option. Accordingly, the molecules were then reacted with $HOPO(OBn)_2$ in the presence of NIS and TMSOTf or TfOH to afford the phosphorylated tri and tetrasaccharides in very good to excellent yields. Unfortunately, complete lack of stereoselectivity was observed in this reaction, resulting in essentially 1:1 mixtures of the corresponding α and β products (Scheme 5).

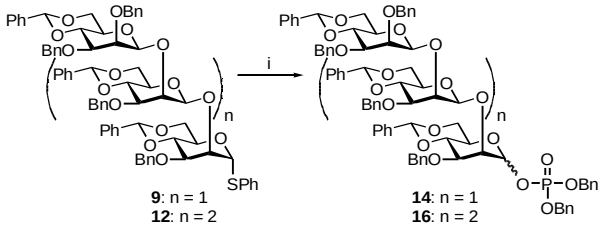
After the phosphorylation step, the protecting groups were then removed in a single reaction in an autoclave reactor. The compounds were dissolved in dry methanol and stirred under 2 bars of H_2 gas overnight in the presence of Pd after which the mixture was filtered through celite (Scheme 6).

Characterization by NMR spectroscopy

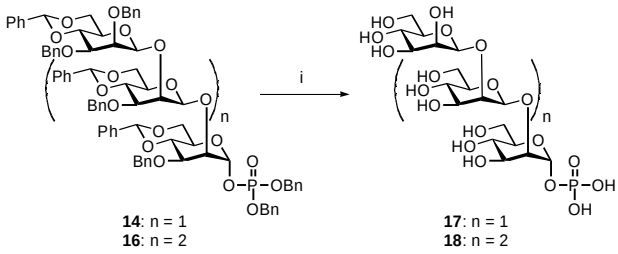
A number of NMR spectroscopic methods were then utilized for characterization and verification of the prepared substrates. Determining the stereochemistry at the anomeric position in mannose by NMR can be challenging due to the H-1 and H-2 protons always occupying either an axial-equatorial (β-mannose) or equatorial-equatorial (α-mannose) position. Consequently, the coupling constant is small in both cases and cannot be utilized as a reliable measure of the anomeric configuration. In contrast, the chemical shift of the H-5 proton appears at a lower frequency in β-linked residues when compared to the corresponding α-compounds.[15] The observed chemical shifts for the trisaccharide **17** were 3.86 ppm (H-5), 3.43 (H-5′) and 3.37 ppm (H-5″). The corresponding values for the tetrasaccharide **18** were 3.84 ppm (H-5), 3.42 ppm (H-5′), 3.39 ppm (H-5″) and 3.38 ppm (H-5‴). The most reliable indicator of the stereochemistry in mannopyranosides is the $^1J_{C\text{-}H}$ coupling constant between the anomeric carbon and the anomeric proton. These values are usually ~170 Hz for the α-anomer and ~160 for the β-anomer.[16] For trisaccharide **17**, the coupling constants were found to be 173.4 Hz ($^1J_{C\text{-}1,\ H\text{-}1}$), 160.5 Hz ($^1J_{C\text{-}1'\text{-}H\text{-}1'}$) and 160.3 Hz ($^1J_{C\text{-}1''\text{-}H\text{-}1''}$). The corresponding values for tetrasaccharide **18** were 172.1 Hz ($^1J_{C\text{-}1,\ H\text{-}1}$), 159.9 Hz ($^1J_{C\text{-}1'\text{-}H\text{-}1'}$), 162.0 Hz ($^1J_{C\text{-}1''\text{-}H\text{-}1''}$) and 160.6 Hz ($^1J_{C\text{-}1'''\text{-}H\text{-}1'''}$), which all are in accordance with the literature values.[16]



Scheme 4. Phosphorylation of compounds **9** and **12** by method 1: (i) NBS, Acetone / H_2O, 0 °C, 1,5 h (**13**: 28 %, **15**: 28); (ii) 1. dibenzyl(*N*,*N*-diisopropyl) phosphoramidite, 1H-tetrazole, CH_2Cl_2, 0 °C 22 h, 2. *m*-CPBA, –60 °C, 2 h, (**14**: 41 %, **16**: 60 %), α : β = 1 : 1 (**14**), α : β = 2 : 1 (**16**).



Scheme 5. Phosphorylation of molecules **9** and **12**, method 2. (i) $HOPO(OBn)_2$, NIS, TfOH (**9**), TMSOTf (**15**), CH_2Cl_2, –20 °C → 0 °C, 1 h (**9**), –50 °C → –20 °C, overnight (**12**) , (**14**: 42 %, **16**: 47 %), α : β ≈ 1 : 1.



Scheme 6. Deprotection of molecules **14** and **16**. (i): H_2, MeOH, Pd/C (10 % w/w), 2 bar, r.t., overnight (**17**: 97 %, **18**: 67 %)

Another problem associated with the NMR spectroscopic characterization of carbohydrates is that most of the carbohydrate protons are surrounded by a very similar chemical and electronic

environment. Therefore, most of the proton signals severely overlap in ^{1}H NMR spectra. 1D-TOCSY (1D-Total Correlation SpectroscopY) can be utilized to overcome these issues and to distinguish between protons from different residues. This reduces the complexity of the oligosaccharide ^{1}H NMR spectrum to monosaccharide level (one residue at a time). After assigning the proton and carbon signals of the individual monosaccharide units by use of standard NMR-techniques (COSY, HSQC, HMBC), the order of residues was then determined from 2D HMBC spectra. In order to obtain accurate chemical shifts and coupling constants, the ^{1}H NMR spectra were simulated with the PERCH (PEak reseaRCH) NMR simulation software.[17]

The accurate coupling constants acquired from the spectral simulations were then used for confirming that the individual mannose units in the molecules reside in $^4C_1$ conformations ($J_{H\text{-}2,\ H\text{-}3} \approx 3.0 - 3.5$ Hz, $J_{H\text{-}3,\ H\text{-}4} \approx 9 - 10$ Hz, $J_{H\text{-}4,\ H\text{-}5} \approx 9 - 10$ Hz). The distances between two axial protons in such conformations are known[18] and can thus be used to calculate all other distances from the intensities of the signals in NOESY and ROESY NMR spectra.

Conformational studies

Already in the 1970s, empirical force fields were used to predict that β-(1→2)-linked mannosides would form crumpled conformations.[19] In previous studies, oligomannosides capped with various alkyl groups at the reducing end, as well as fully unprotected oligomannosides, have been shown to attain helical-like conformations.[19,20] A particular goal of the present work was, therefore, to investigate the influence of the charged phosphate group at the reducing end on the conformational properties of these mannosides, in particular by comparison with the fully deprotected β-(1→2)-mannotetraose investigated by us recently and shown to attain a contorted α-helical conformation in solution.[20] Thus, both the phosphorylated mannotriose and mannotetraose were here modelled and molecular dynamics simulations performed on both compounds in order to elucidate their behaviour in water solution.

Obviously, one single conformation does not correctly represent the molecule in solution, as the saccharide molecules easily rotate and twist around the dihedral angles between the sugar units. Also, the remaining chemical bonds may vibrate and twist around energy minima to produce a large number of different conformations with similar energies. It should be noted that in the NMR experiments, an average of all possible conformations that the molecule can adopt is recorded in a time scale much longer than that used in molecular dynamics simulations (μs vs. fs). High energy conformations exist only for short periods of time and, therefore, correlations of these conformations are not visible unless unrealistically long times are used for NMR experiments.

The definitions of the dihedral angles measured from the molecular dynamics simulations are illustrated in Figure 2, where φ^H_n refers to the angle H1$^{n+\prime}$-C1$^{n+\prime}$-O1$^{n+\prime}$-C2^n and ψ^H_n refers to the angle C1$^{n+\prime}$-O1$^{n+\prime}$-C2^n-H2^n. The lower index *n* shows which glycosidic linkage the angles refer to (i.e., 1, 2 or 3 in the molecules discussed herein). The upper index H will subsequently be omitted, since no other dihedral angles are considered. The carbon and proton atoms of each residue are numbered from 1 to 6 and the different residues distinguished by adding a prime symbol after the number when progressing from the reducing end towards the nonreducing end (the first residue is unprimed, the second has one prime, the third has two etc.).

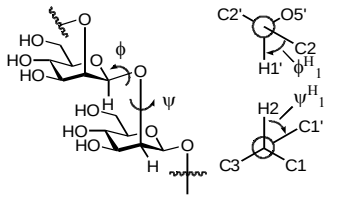


Figure 2. Definitions of the dihedral angles observed during the molecular dynamics simulations.

NOESY and ROESY NMR methods show correlations between protons close to each other in space and can thus be used to verify the modelled conformation. Unfortunately, in the case of NOESY the correlation is often zero for molecules with molecular weights of ~400 – 1500 Da, i.e., from common disaccharides up to pentasaccharide level.[19] To overcome these problems, the NOESY spectrum of the tetrasaccharide was recorded at 5 °C. The ROESY method, however, does not suffer from similar drawbacks and the ROESY spectrum for the trisaccharide was recorded at 25 °C.

In the 2D ROESY spectrum of trisaccharide **17** (Figure 3), the correlations between H1^n and H2$^{n+\prime}$, i.e., correlations across the glycosidic bonds are clearly visible and well separated, which further confirms the correct assignment of the order of residues. Furthermore, a correlation between protons H1 and H1′ is observed. More importantly, correlations between H4 and H1″, as well as correlations between H4 and H2″ are also visible. Such correlations between non-contiguous residues indicate that the structure folds back on itself. Unfortunately, due to overlapping in the spectrum, it is impossible to accurately calculate the distances between protons H4 and H1″ as well as H4 and H2″.

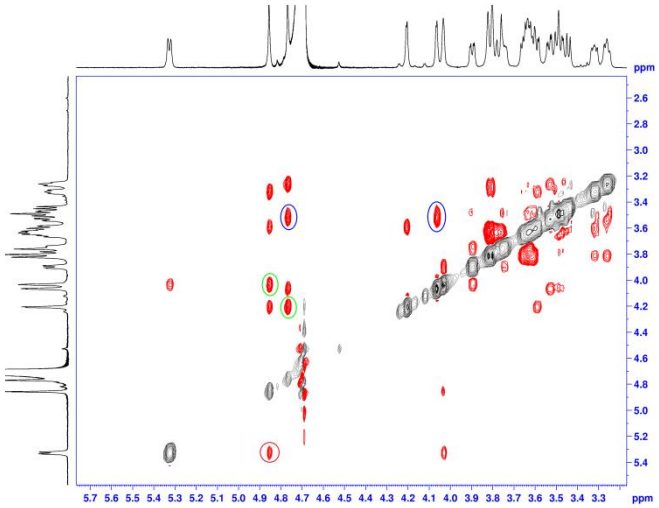


Figure 3. 2D ROESY spectrum of **17**.

Table 1. Interprotonic distances for molecule **17**, measured from the modelled structure as well as calculated from NMR data.

Correlation	Measured Value (A)	Experimental Value (A)
H2, H1′	2.4	2.5
H1, H1′	3.0	2.8
H2′, H1″	2.4	2.4

During the molecular dynamics simulation, the φ angles of trisaccharide **17** remain mostly around 60 °, i.e., in *gauche* conformation, and the ψ angles mostly range from 0 and 30 °

consistent with ~ *eclipsed* conformation. Plotting of these angles further reveals that, during the simulation, a number of minor conformations also appear, although only momentarily. One of the low energy conformations, illustrated in Figure 3, has the angles φ_1 = 54.3 °, ψ_1 = –1.3 °, φ_2 = 44.7 ° and ψ_2 = 25.0 °. From this conformation, some interprotonic distances were measured and are collected in Table 1. The measured values were then compared to values calculated from the intensity of the cross peaks in the ROESY spectrum. The arrows in Figure 4 correspond to the correlations marked with identical colors in Figure 3.

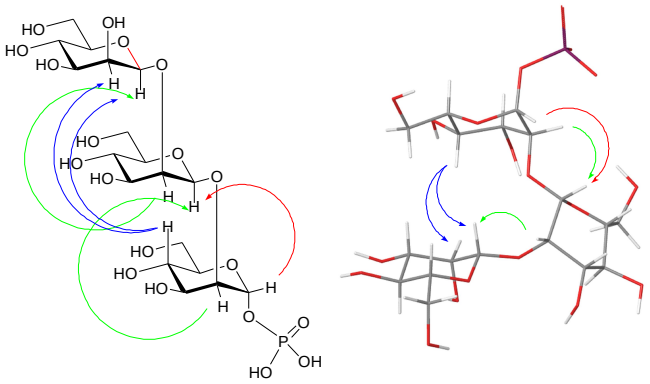


Figure 4. The modelled molecule **17**. The colored arrows indicate ROESY correlations shown in Figure 3.

In the 2D NOESY spectrum of tetrasaccharide **18** (Figure 5), the correlations between protons $H2^n$ and $H1^{n+\prime}$ are visible. In most cases, however, the cross peaks overlap with the correlations between $H1^n$ and $H2^n$ making it impossible to calculate the distances with acceptable precision. Correlations between protons H1 and H1′ as well as those between H1 and H2′ are visible and well separated. Again, correlations can be seen between non-contiguous residues; between H4 and H2″, between H4 and H1‴ and between H4′ and H2‴. Due to overlap of the signals, only the distance between H4′ and H2‴ could be calculated with acceptable precision.

Table 2. Interprotonic distances for molecule **18**, measured from the modelled structure as well as calculated from NMR data.

Correlation	Measured Value (A)	Experimental Value (A)
H1, H2′	3.3	3.3
H1, H1′	2.6	2.6
H4, H2″	3.2	3.0
H4′, H2‴	3.9	3.4[a]

[a] Large deviation probably due to overlapping in the spectrum.

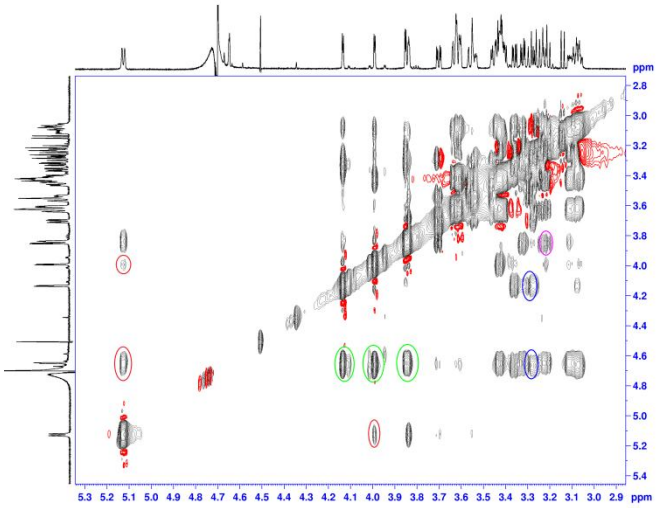


Figure 5. 2D NOESY of spectrum of **18**.

By inspecting the plots of the φ and ψ over the course of the molecular dynamics simulation, it becomes apparent that the tetrasaccharide **18** is somewhat more rigid than the analogous trisaccharide **17**. The glycosidic linkage between the second and third mannose unit is, as expected, more rigid than the two terminal glycosidic linkages. The φ angles appear to remain between approximately 20 and 60 ° and the ψ_1 and ψ_3 angles between 20 and 30 °, respectively. The ψ_2 angle, however, seems to have a quite significant population on both the positive and negative side, around 40 °, indicating that the helical structure can unravel in both directions. One of the low energy conformations, illustrated in Figure 6, contains the angles φ_1 = 26.2 °, ψ_1 = 42.6 °, φ_2 = 23.4 °, ψ_2 = 48.8 °, φ_3 = 36.3 ° and ψ_3 = 23.0 °. Interportonic distances were again measured from this structure and the values compared to those calculated from the NMR data. The results are presented in Table 2. The arrows in Figure 6 correspond to the markings with identical colors in Figure 5

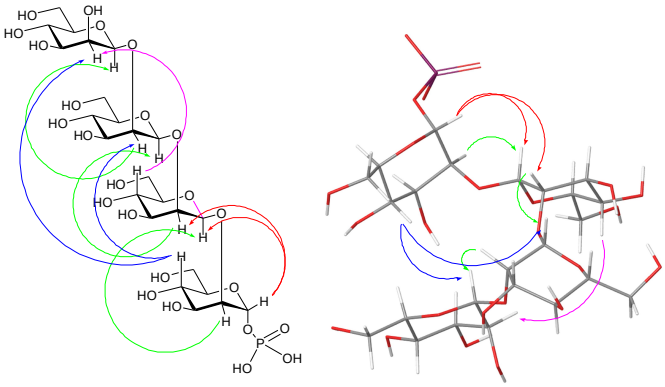


Figure 6. The modelled molecule **18**. The colored arrows indicate ROESY correlations shown in Figure 4.

Inspection of the measured values for both trisaccharide **17** and tetrasaccharide **18** shows good agreement with the experimentally determined values, indicating that the modelled structures correctly represent the actual conformations of the molecules. As emphasized previously, it should be noted that the single low energy conformations depicted in Figures 2 and 4 only represent of the molecules at a specific point in time. By combining the data from the molecular dynamics simulation and the NMR experiments, it is evident that these molecules generally assume

conformations similar to those depicted in Figures 2 and 4, while even fairly large deviations from these conformations may possibly exist for shorter periods of time.

Earlier, it has been suggested that some molecules may be able to form more complex structures in solution through intermolecular stacking phenoma of charged groups such as the phosphate. In principle, such phenomena can be easily followed by NMR-spectroscopy, as the chemical shifts of the atoms involved in the stacking should shift depending on the concentration of the molecule in solution. In order to verify or exclude this possibility for **17** and **18**, NMR samples of both compounds were prepared in three different concentrations (0.1 mM, 0.5 mM and 1.0 mM). ^{1}H NMR spectra were then recorded showing no changes in the corresponding chemical shifts. Likewise, in ^{31}P NMR, no changes were visible in the NMR spectra of the 1.0 mM and 0.5 mM samples with the 0.1 mM samples being omitted due to the unreasonably long time that would have been required for measuring of the ^{31}P NMR spectra. In conclusion, molecules **17** and **18** are not likely to form any larger aggregates in solution.

Conclusions

In this paper, we have for the first time presented the synthesis of phosphate-terminated β-(1→2)-linked mannotriose and mannotetraose. Furthermore it is shown that the charged phosphate group appears to have very little or no effect on the conformational behavior of these molecules as compared to the corresponding unsubstituted β-(1→2)-mannans. Both the phosphorylated trisaccharide and the corresponding tetrasaccharide uptake conformations very similar to their neutrally charged analogues. The underlying reason becomes evident by inspecting the modelled conformations of these compounds, which show that the phosphate group is located outside of the helical structure of the carbohydrate oligomers. Future work with these compounds will involve biological evaluations of the synthesized molecules in anti *C. albicans* assays and comparisons of the results obtained with the parent non-phosphorylated analogues prepared previously at our laboratory. Such studies are important for gaining information on the initiation stages of fungal infections.

Experimental Section

General Considerations. All reagents were purchased from Sigma-Aldrich and used without further purification. Dry solvents were either distilled (CH_2Cl_2 and toluene) or purchased as anhydrous and stored over molecular sieves (DMF and MeOH). TLC was performed on aluminium sheets precoated with Silica gel 60 F_{254} (Merck) and the spots were visualised by UV and charred by using a 1:4 solution of H_2SO_4 in MeOH, followed by heating. Column chromatography was carried out using Silica gel 60 (0.040 – 0.060 mm, Merck). Optical rotations were measured with a Perkin-Elmer 241 polarimeter equipped with a sodium lamp operating at 589 nm, at 24 °C unless mentioned otherwise. HRMS were recorded on a Bruker Micro Q-ToF instrument with electrospray ionisation operating in positive or negative mode. NMR data was recorded using a Bruker Avance spectrometer operating at 600.13 MHz for ^{1}H, 150.90 MHz for ^{13}C and 202.47 MHz for ^{31}P. NOESY spectra of tetrasaccharide **18** were recorded using a Bruker Avance spectrometer with a proton resonance frequency of 700.17 MHz. The complete assignment of proton and carbon spectra was carried out by using a standard set of NMR experiments, ^{1}H NMR, ^{13}C NMR, ^{31}P NMR, DQF-COSY, HSQC (both coupled and decoupled), HMBC and, as previously mentioned, 1D-TOCSY was used to simplify the ^{1}H NMR spectra. In addition, 2D ROESY (for the trisaccharide) and 2D NOESY (for the tetrasaccharide) experiments were performed to assist i^{n} the conformational analysis. Chemical shifts are expressed on the δ (ppm) scale, calibrated using the signals from TMS (tetramethylsilane, δ_H = 0.0 ppm, δ_C = 0.0 ppm) or HOD (δ_H = 4.79 ppm). ^{31}P NMR spectra were calibrated using an external standard, a 0.0485 M solution of PPh_3 in [d_6]acetone, (δ_P = –17.90 ppm). The modelling of the molecules was carried out using Maestro,[21] and the energy minimizations and molecular dynamics simulations with Impact.[22] First, the models were drawn, simply in order to create the atoms and bonds, after which an iterative energy minimization was performed for obtaining a starting point for the dynamics simulation. The acquired models were visually inspected in order to verify that they were realistic. The minimizations and molecular dynamics simulations were performed using the OPLS_2005 force field with a constant dielectricity 80 (the relative permeability for water). The calculations were carried out with continuum solvation with implicit solvent using a surface generalized Born model. The algorithm used for the minimization was a truncated Newton, which is an effective method for acquiring optimized structures. After the minimization, a molecular dynamics simulation was performed in 1 000 000 steps, each 2 fs to obtain a total simulation time of 2 ns. The force field, electrostatic treatment and solvation model were all the similar to those used for the minimization. The equations of motion were integrated using the r-RESPA algorithm, and the structure was stored every fifth step. From the molecular dynamics simulations, the dihedral angles between the mannose units were then recorded and plotted in order to provide a model for flexibility of the model. The low energy conformations obtained by molecular modelling were then compared with the NMR data of these compounds in order to verify that the simulations correctly represent the molecular structures.

General procedure for β-mannosylation. To a solution of the donor (1 equivalent) in dry CH_2Cl_2 (5 ml/0.1 mmol donor) under argon at –60 °C (acetone dry ice) was added pre-activated 4 Å molecular sieves, BSP (1.20 equivalents), TTBP (1.5 equivalents) and Tf_2O (1.3 equivalents). The reaction mixture was stirred for 30 minutes until the activation was complete (confirmed by TLC), after which 1-octene (1 equivalent) was added and the reaction mixture was stirred for another 15 minutes. The reaction mixture was then cooled down to –78 °C and a solution of the acceptor (1.15 equivalents) in dry CH_2Cl_2 (1 ml/0.1 mmol) was added dropwise. The reaction mixture was stirred for 2 h, at –78 °C and, after the reaction was complete, it was quenched by adding triethylphosphite (3 equivalents) and stirred for 1 h. The reaction mixture was then warmed to room temperature and diluted with CH_2Cl_2 (30 ml/100 mg) and washed with a saturated solution of $NaHCO_3$ in H_2O (30 ml/100 g). The water layer was extracted with CH_2Cl_2 (2 × 30 ml/100 mg) after which the combined organic layers were washed with brine (30 ml/100 mg) and dried over Na_2SO_4. The solvent was removed and the crude product was purified by column chromatography to yield the β-coupled product.

General procedure for hydrogenolysis of benzyl and benzylidene protecting groups. To a solution of the protected sugar molecule in dry MeOH (1.5 ml/10 mg protected sugar) was added Pd/C 10% w/w (2 equivalents by mass) and the mixture was stirred in an autoclave under H_2 (2 bar) overnight. The reaction mixture was then filtered through celite and evaporated to dryness to yield the corresponding unprotected product.

Phenyl-2,3-di-*O*-benzyl-4,6-*O*-benzylidene-β-D-mannopyranosyl-(1→2)-3-*O*-benzyl-4,6-*O*-benzylidene-β-D-mannopyranosyl-(1→2)-3-*O*-benzyl-4,6-*O*-benzylidene-1-thio-α-D-mannopyranoside (9). Synthesized from donor **8** (200 mg, 0.2 mmol) and acceptor **6** (107 mg, 0.24 mmol) according to the general procedure for β-mannosylation. The product was purified by column chromatography (hexane : EtOAc 2 : 1, R_f = 0.45) to yield **9** as a white foam. Yield: 166 mg (62%). $[\alpha]_D$ = – 51,5 ° (c = 1.0 CH_2Cl_2, 24 °C). ^{1}H NMR (600.13 MHz, $CDCl_3$, 25 °C): δ = 7.25 – 6.90 (m, 40 H, arom. H), 5.60 (s, 1 H, 4,6-OC*H*′Ph), 5.57 (s, 1 H, 4,6-OC*H*Ph), 5.51 (d, 1 H, $J_{H\text{-}1,\ H\text{-}2}$ = 1.4 Hz, H-1), 5.37 (s, 1 H, 4,6-OC*H*″Ph), 5.10 (d, 1 H, $J_{H\text{-}1'',\ H\text{-}2''}$ = 0.5 Hz, H-1″), 4.97 and 5.72 (each d, each 1 H, *J* = –12.5 Hz, 2-OCH_2Ph), 4.82 and 4.77 (each d, each 1 H, *J* = –12.6 Hz, 3′-OCH_2Ph), 4.67 and 4.62 (each d, each 1 H, *J* = –12.2 Hz, 3-OCH_2Ph), 4.65 (d, 1 H, $J_{H\text{-}1',\ H\text{-}2'}$ = 0.1 Hz, H-1′), 4.56 (dd, 1 H, $J_{H\text{-}2,\ H\text{-}3}$ = 3.0 Hz, H-2), 4.46 and 4.43 (each d,

each 1 H, J = –11.6 Hz, 3″-OCH_2Ph), 4.45 (dd, 1 H, $J_{H\text{-}2'',\ H\text{-}3''}$ = 3.2 Hz, H-2″), 4.36 (dd, 1 H, $J_{H\text{-}2'',\ H\text{-}3'}$ = 3.1 Hz, H-2′) 4.34 (ddd, 1 H, $J_{H\text{-}5,\ H\text{-}6a}$ = 4.9 Hz, $J_{H\text{-}5,\ H\text{-}4}$ = 9.2 Hz, $J_{H\text{-}5,\ H\text{-}6b}$ = 10.2 Hz, H-5), 4.33 (dd, 1 H, $J_{H\text{-}6''a,\ H\text{-}5''}$ = 4.9 Hz, $J_{H\text{-}6''a,\ H\text{-}6''b}$ = –10.5 Hz, H-6″a), 4.31 (dd, 1 H, $J_{H\text{-}6'a,\ H\text{-}5'}$ = 4.8 Hz, $J_{H\text{-}6'a,\ H\text{-}6'b}$ = –10.0 Hz, H-6′a), 4.23 (dd, 1 H, $J_{H\text{-}6a,\ H\text{-}6b}$ = –10.3 Hz, H-6a), 4.22 (dd, 1 H, $J_{H\text{-}4'',\ H\text{-}5''}$ = 9.2 Hz, $J_{H\text{-}4'',\ H\text{-}3''}$ = 9.9 Hz, H-4″), 4.06 (dd, 1 H, $J_{H\text{-}4',\ H\text{-}5'}$ = 9.3 Hz, $J_{H\text{-}4',H\text{-}3'}$ = 9.8 Hz, H-4′), 3.99 (dd, 1 H, $J_{H\text{-}4,\ H\text{-}3}$ = 10.1 Hz, H-4), 3.98 (dd, 1 H, H-3), 4.97 (dd, 1 H, $J_{6''b,\ 5''}$ = 10.1 Hz, H-6″b), 3.77 (dd, 1 H, $J_{H\text{-}6b,\ H\text{-}5}$ = 10.2 Hz, H-6b), 3.72 (dd, $J_{H\text{-}6'b,\ H\text{-}5'}$ = 10.1 Hz, H-6′b), 3.64 (dd, 1 H, H-3′), 3.55 (dd, 1 H, H-3″), 3.43 (ddd, 1 H, H-5″), 3.33 (ddd, 1 H, H-5′) ppm.

^{13}C NMR (150.9 MHz, $CDCl_3$, 25 °C): δ = 139.4 – 126.0 (arom. C), 103.0 (C-1″), 102.0 (4,6-OC*H*Ph), 101.6 (4,6-OC″HPh), 101.3 (4,6-OC′HPh), 98.5 (C-1′), 85.6 (C-1), 79.3 (C-3″), 79.0 (C-4), 78.2 (C-4′), 78.1 (C-4″), 76.2 (C-2′ and C-2″), 75.7 (C-3′), 74.9 (2-OCH_2Ph), 74.8 (C-2), 74.6 (C-3), 72.2 (3″-OCH_2Ph), 71.8 (3-OCH_2Ph), 71.0 (3′-OCH_2Ph), 68.7 (C-6″), 68.6 (C-6 and C-6′), 67.9 (C-5″), 67.8 (C-5′), 65.2 (C-5) ppm.

HRMS: *m/z* calcd. for $C_{73}H_{73}O_{15}SNa$ [M+ Na]$^+$: 1243.4490; Found 1243.4502

2,3-Di-*O*-benzyl-4,6-*O*-benzylidene-β-D-mannopyranosyl-(1→2)-3-*O*-benzyl-4,6-*O*-benzylidene-β-D-mannopyranosyl-(1→2)-3-*O*-benzyl-4,6-*O*-benzylidene-D-mannopyranose (13). A solution of **9** (255 mg, 1 equivalent) in 5 ml of a 50:1 mixture of acetone : H_2O was cooled on an ice bath and NBS (69 mg, 2 equivalents) was added and the reaction mixture was stirred ad 0 °C for 30 min after which additional NBS (35 mg, 1 equivalent) was added and the reaction mixture was again stirred for 30 min. Additional NBS (69 mg 2 equivalents) was added and the reaction mixture was stirred for 30 min after which solid $Na_2S_2O_3$ was added until the yellow color disappeared. The solvent was evaporated and the resulting oil was dissolved in 50 ml CH_2Cl_2 and washed with 3 × 20 ml H_2O and dried over Na_2SO_4 after which the solvent was removed. The crude product was purified by column chromatography (hexane : EtOAc 1 : 1, R_f = 0.36) to yield **13** as a white foam. Yield: 61 mg (28%). Due to the complexity of the NMR spectra caused by a mixture of anomers, the spectra were not fully assigned.

HRMS: *m/z* calcd. for $C_{67}H_{68}O_{16}Na$ [M + Na]$^+$: 1151.4405; Found 1151.4401

[2,3-Di-*O*-benzyl-4,6-*O*-benzylidene-β-D-mannopyranosyl-(1→2)-3-*O*-benzyl-4,6-*O*-benzylidene-β-D-mannopyranosyl-(1→2)-3-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranosyl] dibenzylphosphate (14). Method 1: To a solution of **13** (40 mg, 1 equivalent) in dry CH_2Cl_2 under argon was added 1H-tetrazole (9.3 mg, 3.8 equivalents) after which the reaction mixture was cooled down to 0 °C. Dibenzyl(*N*,*N*-diisopropyl) phosphoramidite (30 µl, 2.5 equivalents) was added after which the reaction mixture was allowed to warm up to room temperature and was stirred for 22 h. The reaction mixture was then cooled to –60 °C and 20 mg *m*-CPBA was added. The reaction mixture was stirred at 0 °C for 1.5 h and at room temperature for 2 h after which it was diluted with 40 ml CH_2Cl_2, washed with 2 × 15 ml of a saturated solution of $Na_2S_2O_3$ in H_2O, 2 × 15 ml of a saturated solution of $NaHCO_3$ in H_2O and 2 × 10 ml H_2O. The organic layer was purified by column chromatography (hexane : EtOAc 1 : 1, R_f = 0.57) to yield **14** as a white foam. Yield of α-product: 20 mg (41%). $[\alpha]_D$ = – 51,7 ° (c = 0.1 CH_2Cl_2, 24 °C).

Method 2: To a solution of **9** (140 mg, 1 equivalent) in 3 ml dry CH_2Cl_2 under argon was added $HOPO(OBn)_2$ (94.3 mg, 3 equivalents) and 4 Å molecular sieves. The reaction mixture was cooled to –20 °C and NIS (30.5 mg, 1.2 equivalents) and TfOH, (2.5 µl, 0.12 equivalents) were added. The reaction mixture was stirred at 0 °C for 1 h after which the reaction was quenched by adding 0.5 ml pyridine. The reaction mixture was diluted with 20 ml CH_2Cl_2 and washed with 2 × 15 ml of a saturated solution of $Na_2S_2O_2$ in H_2O, 2 × 15 ml of a saturated solution of $NaHCO_3$ in H_2O and 1 × 15 ml H_2O. The organic layer was dried over Na_2SO_4 and the solvent was removed. The crude product was purified by column chromatography (hexane : EtOAc 1 : 1) to yield **14** as a white foam. Yield of α-product: 64.2 mg (42 %). ^{1}H NMR (600.13 MHz, $CDCl_3$, 25 °C): δ = 7.60 – 7 (m, 45 H, arom. H), 5.60 (s, 1 H, 4,6-OC*H*″Ph), 5.58 (dd, 1 H, $J_{H\text{-}1,\ H\text{-}2}$ = 1.5 Hz, $J_{H\text{-}1,\ P}$ = 6.2 Hz), 5.45 (s, 1 H, 4,6-OC*H*′Ph), 5.33 (s, 1 H, 4,6-OC*H*Ph), 5.08 and 5.03 [each dd, each, 1 H, J = –11.9 Hz, $J_{CH2a,P}$ = 8.9 Hz, $J_{CH2b,P}$ = 8.2 Hz, PO(OCH_2Ph)], 5.02 and 4.99 [each dd, each, 1 H, J = –11.8 Hz, $J_{CH2a,\ P}$ = 13.6 Hz, $J_{CH2b,P}$ = 9.6 Hz, PO(OCH_2Ph)], 5.00 (d, 1 H, $J_{H\text{-}1'',\ H\text{-}2''}$ = 0.1 Hz, H-1″), 4.91 and 4.69 (each d, each 1 H, J = –12.5 Hz, 2″-OCH_2Ph), 4.83 and 4.78 (each d, each 1 H, J = –12.5 Hz, 3′-OCH_2Ph), 4.60 and 4.56 (each d, each 1 H, J = –12.2 Hz, 3-OCH_2Ph), 4.47 (s, 1 H, H-1′), 4.45 and 4.42 (each d, each 1 H, J = –11.7 Hz, 3″-OCH_2Ph), 4.39 (dd, 1 H, $J_{H\text{-}2'',\ H\text{-}3''}$ = 3.2 Hz, H-2″), 4.34 (dd, 1 H, $J_{H\text{-}6''a,\ H\text{-}5''}$ = 4.8 Hz, $J_{H\text{-}6''a,\ H\text{-}6''b}$ = –10.4 Hz, H-6″a), 4.29 (d, 1 H, $J_{H\text{-}2',\ H'3'}$ = 3.1 Hz, H-2′), 4.26 (dd, 1 H, $J_{H\text{-}6'a,\ H\text{-}5'}$ = 4.8 Hz, $J_{H\text{-}6'a,\ H\text{-}6'b}$ = –10.2 Hz, H-6′a), 4.22 (dd, 1 H, $J_{H\text{-}4'',\ 5''}$ = 9.2 Hz, $J_{H\text{-}4'',\ H\text{-}3''}$ = 9.8 Hz, H-4″), 4.15 (dd, 1 H, $J_{H\text{-}2,\ H\text{-}3}$ = 3.3 Hz, H-2), 4.02 (dd, 1 H, $J_{H\text{-}4',\ H\text{-}5'}$ = 9.4 Hz, $J_{H\text{-}4',\ H\text{-}3'}$ = 9.7 Hz, H-4′), 3.99 (dd, 1 H, $J_{H\text{-}6a,\ H\text{-}5}$ = 4.9 Hz, $J_{H\text{-}6a,\ H\text{-}6b}$ = –10.3 Hz, H-6a), 3.97 (dd, 1 H, $J_{H\text{-}6''b,\ 5''}$ = 10.2 Hz, H-6″b), 3.85 (dd, 1 H, $J_{H\text{-}4,H\text{-}5}$ = 9.0 Hz, $J_{H\text{-}4,H\text{-}3}$ = 10.2 Hz, H-4), 3.84 (ddd, 1 H, $J_{H\text{-}5,\ H\text{-}6b}$ = 10.1 Hz, H-5), 3.83 (dd, 1 H, H-3), 3.67 (dd, 1 H, $J_{H\text{-}6'b,\ H\text{-}5'}$ = 10.1 Hz, H-6′b), 3.60 (dd, 1 H, H-6b), 3.59 (dd, 1 H, H-3′), 3.53 (dd, 1 H, -3″), 3.41 (ddd, 1 H, H-5″), 3.24 (ddd, 1 H, H-5″) ppm.

^{13}C NMR (150.9 MHz, $CDCl_3$, 25 °C): δ = 139.3 – 135.2(arom. C), 103.0 (C-1″), 101.9 (4,6-OC′HPh), 101.6 (6,6-OCHPh), 101.3 (4,6-OC″HPh), 99.6 (C-1′), 95.7 ($^2J_{C\text{-}1,P}$ = 5.7 Hz, C-1), 79.2 (C-3″), 78.1 (C-4, C-4′, C-4″), 76.2 (C-2′), 76.0 (C-2″), 75.5 (C-3′), 74.9 (2-OCH_2Ph), 73.7 ($^3J_{C\text{-}2,P}$ = 9.9 Hz, C-2), 73.5 (C-3), 72.1 (3″-OCH_2Ph), 71.9 (3-OCH_2Ph), 71.1 (3′-OCH_2Ph), 69.9 [$^2J_{C,P}$ = 6.1 Hz, PO(OCH_2Ph)], 69.8 ($^2J_{C,P}$ = 6.1 Hz, PO(OCH_2Ph)], 68.7 (C-6″), 68.5 (C-6′), 68.3 (C-6), 67.9 (C-5″), 67.6 (C-5′), 65.5 (C-5) ppm.

^{31}P NMR (242.9 MHz, $CDCl_3$, 25 °C): δ = –2.88 ppm.

HRMS: *m/z* calcd. for $C_{81}H_{81}O_{19}PNa$ [M+ Na]$^+$: 1411.5007; Found 1411.5025

β-D-Mannopyranosyl-(1→2)-β-D-mannopyranosyl-(1→2)-α-D-mannopyranosylphosphate (17). Synthesized from **14** (22 mg, 0.016 mmol) according to the general procedure for hydrogenolysis of benzyl and benzylidene protecting group. Filtration through celite with water yielded the product as a colorless oil. Yield: 9 mg (97%). $[\alpha]_D$ = – 13 ° (c = 0.01 H_2O, 24 °C). ^{1}H NMR (600.13 MHz, $CDCl_3$, 25 °C): δ = 5.45 (dd, 1 H, $J_{H\text{-}1,\ H\text{-}2}$ = 1.5 Hz, $J_{H\text{-}1,\ P}$ = 7.9 Hz, H-1), 4.96 (d, 1 H, $J_{H\text{-}1',\ H\text{-}2'}$ = 0.2 Hz, H-1′), 4.88 (d, 1 H, $J_{H\text{-}1'',\ H\text{-}2''}$ = 0.5 Hz, H-1″), 4.32 (dd, 1 H, $J_{H\text{-}2',\ H\text{-}3'}$ = 3.4 Hz, H-2′), 4.18 (dd, 1 H, $J_{H\text{-}2'',\ H\text{-}3''}$ = 3.3 Hz, H-2″), 4.15 (dd, $J_{H\text{-}2,\ H\text{-}3}$ = 3.1 Hz, H-2), 4.01 (dd, $J_{H\text{-}3,\ H\text{-}4}$ = 9.8 Hz, H-3), 3.93 (dd, 2 H, $J_{H\text{-}6'a,\ H\text{-}5'}$ = 2.1 Hz, $J_{H\text{-}6'a,\ H\text{-}6'b}$ = –11.9 Hz, $J_{H\text{-}6''a,\ H\text{-}5''}$ = 2.2 Hz, $J_{H\text{-}6''a,\ H\text{-}6''b}$ = –12.6 Hz, H-6′a and H-6″a), 3.89 (dd, 1 H, $J_{H\text{-}6a,\ H\text{-}5}$ = 2.1 Hz, $J_{H\text{-}6''a,\ H\text{-}6''b}$ = –12.4 Hz, H-6a), 3.86 (ddd, $J_{H\text{-}5,\ H\text{-}6b}$ = 5.6 Hz, $J_{H\text{-}5,\ H\text{-}4}$ = 9.8 Hz, H-5), 3.77 (dd, 1 H, H-6b), 3.75 (dd, 1 H, $J_{H\text{-}6'b,\ H\text{-}5'}$ = 5.9 Hz, H-6′b), 3.73 (dd, 1 H, $J_{H\text{-}6''b,\ H\text{-}5''}$ = 6.4 Hz, H-6″b), 3.70 (dd, 1 H, $J_{H\text{-}3',\ H\text{-}4'}$ = 9.9 Hz, H-3′), 3.64 (dd, 1 H, $J_{H\text{-}3'',\ H\text{-}4''}$ = 9.9 Hz, H-3″), 3.61 (each dd, each 1 H, $J_{H\text{-}4',\ H\text{-}5'}$ = 9.7 Hz, H-4 and H-4′), 3.57 (dd, 1 H, $J_{H\text{-}4'',\ H\text{-}5''}$ = 9.8 Hz, H-4″), 3.43 (ddd, 1 H, H-5′), 3.37 (ddd, 1 H, H-5″) ppm.

^{13}C NMR (150.9 MHz, $CDCl_3$, 25 °C): δ = 101.0 (C-1″), 98.9 (C-1′), 92.9 ($^2J_{C\text{-}1,\ P}$ = 4.0 Hz, C-1), 78.8 ($^3J_{C\text{-}2,\ P}$ = 6.6 Hz, C-2), 78.7 (C-2′), 76.2 (C-5″), 76.1 (C-5′), 72.9 (C-5), 72.8 (C-3″), 72.1 (C-3′), 70.4 (C-2″), 69.1 (C-3), 67.3 and 66.9 (C-4 and C-4′), 66.7 (C-4″), 61.0 (C-6″), 60.7 (C-6′), 60.5 (C-6) ppm.

^{31}P NMR (242.9 MHz, $CDCl_3$, 25 °C): δ = 1.46 ppm.

HRMS: *m/z* calcd. for $C_{18}H_{32}O_{19}P$ [M - H]$^-$: 853.1276; Found 853.1252

Phenyl-2-*O*-(4-methoxybenzyl)-3-*O*-benzyl-4,6-*O*-benzylidene-β-D-mannopyranosyl-(1→2)-3-*O*-benzyl-4,6-*O*-benzylidene-1-thio-α-D-mannopyranoside (10). Synthesized from donor **7** (500 mg 0.876 mmol) and acceptor **6** (455 mg 1.01 mmol) according to the general procedure for β-mannosylation. The crude product was purified by column chromatography (hexane : EtOAc 2 : 1, R_f = 0.45) Yield: 455 mg (57%). $[\alpha]_D$ = – 52,5 ° (c = 1.0 CH_2Cl_2, 24 °C). ^{1}H NMR (600.13 MHz, $CDCl_3$, 25 °C): δ = 7.75 – 7.20 (m, 29 H, arom. H), 5.59 (s, 1 H, 4,6-OC*H*′Ph), 5.52 (s, 1 H, 4,6-OC*H*Ph), 5.50 (d, 1 H, $J_{H\text{-}1,\ H\text{-}2}$ = 1.5 Hz, H-1), 4.97 and 4.89 (each d, each 1 H, J = –11.8, 2′-OCH_2Ph), 4.80 and 4.75 (each d, each 1 H, J = –

12.1 Hz, 3-OCH_2Ph), 4.67 and 4.59 (each d, each 1 H, J = –12.5 Hz, 3′-OCH_2Ph), 4.61 (d, 1 H, $J_{H\text{-}1',\ H\text{-}2'}$ = 0.9 Hz, H-1′), 4.51 (dd, 1 H, $J_{H\text{-}2,\ H\text{-}3}$ = 3.2 Hz, H-2), 4.33 (ddd, 1 H, $J_{H\text{-}5,\ H\text{-}6a}$ = 4.8 Hz, $J_{H\text{-}5,\ H\text{-}4}$ = 9.5 Hz, $J_{H\text{-}5,\ H\text{-}6b}$ = 10.2 Hz, H-5), 4.24 (dd, 2 H, $J_{H\text{-}6a,\ H\text{-}6b}$ = –10.2 Hz, $J_{H\text{-}6'a,\ H\text{-}5'}$ = 4.9 Hz, $J_{H\text{-}6'a,\ H\text{-}6'b}$ = –10.4 Hz, H-6a and H-6b), 4.23 (dd, 1 H, $J_{H\text{-}4',\ H\text{-}5'}$ = 9.3 Hz, $J_{H\text{-}4',\ H\text{-}3'}$ = 9.9 Hz, H-4′), 4.17 (dd, 1 H, $J_{H\text{-}4,\ H\text{-}3}$ = 10.0 Hz, H-4), 3.98 (dd, 1 H, H-3), 3.96 (dd, 1 H, $J_{H\text{-}2',\ H\text{-}3'}$ = 3.2 Hz, H-2′), 3.82 (dd, $J_{H\text{-}6'b,\ H\text{-}5'}$ = 10.1 Hz, H-6′b), 3.79 (dd, 1 H, H-6b), 3.73 (s, 3 H, OCH_3), 3.57 (dd, 1 H, H-3′), 3.29 (ddd, 1 H, H-5) ppm.

^{13}C NMR (150.9 MHz, $CDCl_3$, 25 °C): δ = 159.1 – 113.5 (arom. C), 101.7 (4,6-O*C*HPh), 101.4 (4,6-O*C*′HPh), 99.8 (C-1′), 86.4 (C-1), 78.7 (C-4), 78.4 (C-4′), 77.5 (C-3′), 76.1 (C-2), 75.4 (C-2′), 74.3 (C-3), 74.2 (2′-O*C*H_2Ph), 72.2 (3′-O*C*H_2Ph), 71.4 (3-O*C*H_2Ph), 68.6 (C-6), 68.5 (C-6′), 67.7 (C-5′), 65.4 (C-5), 55.2 (O*C*H_3) ppm.

HRMS: *m/z* calcd. for $C_{54}H_{54}O_{11}SNa$ $[M + Na]^+$: 933.3285; Found 933.3228; *m/z* calcd. for $C_{54}H_{58}O_{11}SN$ $[M + NH_4]^+$: 928.3731; Found 928.3634.

Phenyl-3-*O*-benzyl-4,6-*O*-benzylidene-β-D-mannopyranosyl-(1→2)-3-*O*-benzyl-4,6-*O*-benzylidene-1-thio-α-D-mannopyranoside (11). A solution of **10** (200 mg, 1 equivalent) in 3 ml CH_2Cl_2 was cooled down to 0 °C and a suspension of DDQ (74 mg, 1.5 equivalents) in 3 ml H_2O was added dropwise. The reaction mixture was stirred for 1 h at 0 °C after which it was diluted with 25 ml CH_2Cl_2 and washed with 2 × 25 ml of a saturated solution of $NaHCO_3$ in H_2O. The water layer was extracted with 2 × 25 ml CH_2Cl_2, dried over Na_2SO_4 and evaporated to dryness. The crude product was purified by column chromatography (hexane : EtOAc 2 : 1) to yield the **11** as a white foam. Yield: 153 mg (88%). NMR spectra are in accordance with those published previously.[14]

Phenyl-2,3-di-*O*-benzyl-4,6-*O*-benzylidene-β-D-mannopyranosyl-(1→2)-3-*O*-benzyl-4,6-*O*-benzylidene-β-D-mannopyranosyl-(1→2)-3-*O*-benzyl-4,6-*O*-benzylidene-β-D-mannopyranosyl-(1→2)-3-*O*-benzyl-4,6-*O*-benzylidene-1-thio-α-D-mannopyranoside (12). Synthesized from donor **8** (290 mg, 0.33 mmol) and acceptor **11** (300 mg, 0.38 mmol) according to the general procedure for β-mannosylation. The crude product was purified by column chromatography (hexane : EtOAc 2 : 1, R_f (R kursivt på alla platser)= 0.50). Yield: 182 mg (35 %). $[\alpha]_D$ = – 62,0 ° (c = 1.0 CH_2Cl_2, 24 °C). ^{1}H NMR (600.13 MHz, $CDCl_3$, 25 °C): δ = 7.75 – 7.00 (m, 50 H, arom. H), 5.57 (s, 1 H, 4,6-OC*H*″Ph), 5.54 (s, 1 H, 4,6-OC*H*Ph), 5.49 (d, 1 H, $J_{H\text{-}1,\ H\text{-}2}$ = 1.3 Hz, H-1), 5.44 (s, 1 H, 4,6-OC*H*′Ph), 5.40 (s, 1 H, 4,6-OC*H*‴Ph), 5.27 (s, 1 H, H-1″), 5.07 (d, 1 H, $J_{H\text{-}1''',\ H\text{-}2'''}$ = 0.1 Hz, H-1‴), 5.00 and 4.84 (each d, each 1 H, J = –12.6 Hz, 2‴-OCH_2Ph), 4.70 (s, 2 H, 3-OCH_2Ph), 4.68 and 4.61 (each d, each 1 H, J = –12.4 Hz, 3′-OCH_2Ph), 4.68 (s, 1 H, H-1′), 4.62 and 4.44 (each d, each 1 H, J = –11.9 Hz, 3″-OCH_2Ph), 4.54 (d, 1 H, $J_{H\text{-}2'',\ H\text{-}3''}$ = 3.0 Hz, H-2″), 4.53 (dd, 1 H, $J_{H\text{-}2,\ H\text{-}3}$ = 3.1 Hz, H-2), 4.50 and 4.40 (each d, each 1 H, J = –11.8 Hz, 3‴-OCH_2Ph), 4.48 (d, 1 H, $J_{H\text{-}2',\ H\text{-}3'}$ = 3.4 Hz, H-2′), 4.41 (dd, 1 H, $J_{H\text{-}2''',\ H\text{-}3'''}$ = 3.3 Hz, H-2‴), 4.33 (dd and ddd, 2 H, $J_{H\text{-}6''a,\ H\text{-}5''}$ = 4.8 Hz, $J_{H\text{-}6''a,\ H\text{-}6''b}$ = –10.2 Hz, $J_{H\text{-}5,\ H\text{-}6a}$ = 5.0 Hz, $J_{H\text{-}5,\ H\text{-}4}$ = 9.2 Hz, $J_{H\text{-}5,\ H\text{-}6b}$ = 10.2 Hz, H-6″a and H-5), 4.31 (dd, 1 H, $J_{H\text{-}6'a,\ H\text{-}5'}$ = 4.8 Hz, $J_{H\text{-}6'a,\ H\text{-}6'b}$ = –10.3 Hz), 4.23 (dd, 1 H, $J_{H\text{-}6a,\ H\text{-}6b}$ = –10.3 Hz, H-6a), 4.19 (dd and dd, 2 H, $J_{H\text{-}4''',\ H\text{-}5'''}$ = 9.3 Hz, $J_{H\text{-}4''',\ H\text{-}3'''}$ = 9.9 Hz, $J_{H\text{-}6'''a,\ H\text{-}5'''}$ = 4.9 Hz, $J_{H\text{-}6'''a,\ H\text{-}6'''b}$ = –10.3 Hz, H-4‴ and H-6‴a), 4.06 (dd, 1 H, $J_{H\text{-}4'',\ H\text{-}5''}$ = 9.3 Hz, $J_{H\text{-}4'',\ H\text{-}3''}$ = 9.8 Hz, H-4″), 3.99 (dd, 1 H, $J_{H\text{-}4,\ H\text{-}3}$ = 9.9 Hz, H-4), 3.97 (dd, 1 H, H-3), 4.93 (dd, 1 H, $J_{H\text{-}4',\ H\text{-}5'}$ = 9.3 Hz, $J_{H\text{-}4',\ H\text{-}3'}$ = 9.8 Hz, H-4′), 3.87 (dd, 1 H, $J_{H\text{-}6''b,\ H\text{-}5'''}$ = 10.1 Hz, H-6‴b), 3.78 (dd, 1 H, $J_{H\text{-}6''b,\ H\text{-}5''}$ = 10.1 Hz, H-6″b), 4.77 (dd, 1 H, $J_{H\text{-}6'b,\ H\text{-}5'}$ = 10.1 Hz, H-6′b), 3.74 (dd, 1 H, H-6b), 3.66 (dd, 1 H, H-3′), 5.54 (dd, 1 H, H-3‴), 3.53 (dd, 1 H, H-3″), 3.43 (ddd, 1 H, H-5″), 3.36 (ddd, 1 H, H-5′), 3.32 (ddd, 1 H, H-5‴) ppm.

^{13}C NMR (150.9 MHz, $CDCl_3$, 25 °C): δ = 139.4 – 126.1 (arom. C), 103.4 (C-1‴), 102.1 (4,6-O*C*HPh), 102.0 (4,6-O*C*′HPh), 101.8 (C-1″), 101.5 (4,6-O*C*‴HPh), 101.3 (4,6-O*C*″HPh), 99.1 (C-1′), 85.7 (C-1), 79.1 (C-4), 79.0 (C-3‴), 78.4 (C-4′), 78.3 (C-4‴), 78.2 (C-4″), 77.0 (C-3″), 76.3 (C-3′), 76.1 (C-2″), 76.0 (C-2‴), 75.5 (C-2), 74.7 (2‴-O*C*H_2Ph), 74.5 (C-3), 74.0 (C-2′), 72.2 (3‴-O*C*H_2Ph), 72.1 (3-O*C*H_2Ph), 71.3 (3′-O*C*H_2Ph), 70.9 (3″-O*C*H_2Ph), 68.8 (C-6‴), 68.7 (C-6″, C-6′), 68.6 (C-6), 68.0 (C-5″), 67.9 (C-5′), 67.5 (C-5‴), 65.2 (C-5) ppm.

HRMS: *m/z* calcd. for $C_{93}H_{92}O_{20}SNa$ $[M + Na]^+$: 1583.5800; Found 1583.5812.

2,3-Di-*O*-benzyl-4,6-*O*-benzylidene-β-D-mannopyranosyl-(1→2)-3-*O*-benzyl-4,6-*O*-benzylidene-β-D-mannopyranosyl-(1→2)-3-*O*-benzyl-4,6-*O*-benzylidene-β-D-mannopyranosyl-(1→2)-3-*O*-benzyl-4,6-*O*-benzylidene-D-mannopyranose (15). A solution of **12** (250 mg, 1 equivalent) in 5 ml of a 6:1 mixture of acetone : H_2O was cooled on an ice bath and NBS (60 mg, 2 equivalents) was added and the reaction mixture was stirred for 30 min. Additional NBS (30 mg, 1 equivalent) was added and the reaction mixture was again stirred for 30 min after which additional NBS (30 mg, 1 equivalent) was added and the reaction mixture was stirred for 30 min. The reaction was quenched by adding solid $Na_2S_2O_3$ until the yellow color had disappeared. The solvent was evaporated and the residue was dissolved in 50 ml CH_2Cl_2 and washed with 2 × 20 ml H_2O. The organic layer was dried over Na_2SO_4 and evaporated to dryness. The crude product was purified by column chromatography (hexane : EtOAc 1 : 1, R_f = 0.38) to yield **15** as a white foam. Yield (62 mg (28%). Due to the complexity of the NMR spectra caused by a mixture of anomers, the spectra were not fully assigned.

HRMS: *m/z* calcd. for $C_{87}H_{92}O_{21}N$ $[M + NH_4]^+$: 1486.6162; Found 1486.6174.

[2,3-Di-*O*-benzyl-4,6-*O*-benzylidene-β-D-mannopyranosyl-(1→2)-3-*O*-benzyl-4,6-*O*-benzylidene-β-D-mannopyranosyl-(1→2)-3-*O*-benzyl-4,6-*O*-benzylidene-β-D-mannopyranosyl-(1→2)-3-*O*-benzyl-4,6-*O*-benzylidene-D-mannopyranosyl] dibenzylphosphate (16). Method 1: To a solution of **15** (35 mg, 1 equivalent) in 4 ml dry CH_2Cl_2 under argon was added 1H-tetrazole (6.3 mg, 3.8 equivalents) after which the solution was cooled down to 0 °C. Dibenzyl(*N*,*N*-diisopropyl) phosphoramidite (22 µl, 2.5 equivalents) was added and the reaction mixture was allowed to return to room temperature and was stirred for 2 h. The reaction mixture was cooled down to –60 °C and *m*-CPBA (13.5 mg, 3.8 equivalents) was added and the mixture was stirred at 0 °C for 1 h and then at room temperature for 1 h. The reaction mixture was diluted with 50 ml CH_2Cl_2 and washed with 2 × 15 ml of a saturated solution of $Na_2S_2O_3$ in H_2O, 2 × 15 ml of a saturated solution of $NaHCO_3$ in H_2O and 2 × 10 ml H_2O after which the organic layer was dried over Na_sSO_4 and concentrated to dryness. The crude product was purified by column chromatography (hexane : EtOAc 1 : 1, R_f = 0.57) Yield of α-product: 25 mg (60 %). $[\alpha]_D$ = – 61,2 ° (c = 0,1 CH_2Cl_2, 24 °C).

Method 2: To a solution of **12** (50 mg, 1 equivalent) in 3 ml dry CH_2Cl_2 under argon was added $PO(OBn)_2Oh$ (26.7 mg, 3 equivalents) and 4 Å molecular sieves. The reaction mixture was cooled down to –50 °C and NIS (8.6 mg, 1.2 equivalents) and TMSOTf (0.7 µl, 0.12 equivalents) were added. The reaction mixture was stirred at –50 °C for 1 h and warmed to –20 °C and stirred for 3.5 h after which additional TMSOTf (0.7 µl, 0.12 equivalents) was added and the reaction mixture was stirred for another 17.5 h at –20 °C. The reaction was quenched by adding 0.5 ml pyridine after which the reaction mixture was diluted with 20 ml CH_2Cl_2 and washed with 2 × 15 ml of a saturated solution of $Na_2S_2O_3$ in H_2O, 2 × 15 ml of a saturated solution of $NaHCO_3$ in H_2O and 1 × 15 ml H_2O. The organic layer was dried over Na_2SO_4 and evaporated to dryness. The crude product was purified by column chromatography (hexane : EtOAc 1:1). Yield of α-product: 26 mg (47%). ^{1}H NMR (600.13 MHz, $CDCl_3$, 25 °C): δ =7.75 – 6.75 (m, 55 H, arom. H), 5.59 (dd, 1 H, $J_{H\text{-}1,\ H\text{-}2}$ = 1.7 Hz, $J_{H\text{-}1,\ P}$ = 6.3 Hz, H-1), 5.56 (s, 1 H, 4,6-OC*H*‴Ph), 5.43 (s, 1 H, 4,6-OC*H*Ph), 5.41 (s and s, 2 H, 4,6-OC*H*′Ph and 4,6-OC*H*″Ph), 5.14 (d, 1 H, $J_{H\text{-}1'',\ H\text{-}2''}$ = 0.1 Hz, H-1″), 5.08 and 5.02 [each dd, each, 1 H, J = –11.9 Hz, $J_{CH2a,P}$ = 9.1 Hz, $J_{CH2b,P}$ = 8.2 Hz, PO(OCH_2Ph)], 5.06 (d, 1 H, $J_{H\text{-}1''',\ H\text{-}2'''}$ = 0.1 Hz, H-1‴), 5.01 and 4.98 [each dd, each, 1 H, J = –11.6 Hz, $J_{CH2a,P}$ = 8.4 Hz, $J_{CH2b,P}$ = 9.9 Hz, PO(OCH_2Ph)], 4.99 and 4.83 (each d, each 1 H, J = –12.5 Hz, 2‴-OCH_2Ph), 4.70 and 4.63 (each d, each 1 H, J = –12.4 Hz, 3′-OCH_2Ph), 4.65 and 4.50 (each d, each 1 H, J = –11.9 Hz, 3″-OCH_2Ph), 4.64 and 4.61 (each

d, each 1 H, J = –12.4 Hz, 3-OCH_2Ph), 4.51 (dd, $J_{H\text{-}2'',\ H\text{-}3''}$ = 3.1 Hz, H-2″), 4.50 (d, 1 H, $J_{H\text{-}1',\ H\text{-}2'}$ = H-1′), 4.50 and 4.38 (each d, each 1 H, J = –11.8 Hz, 3‴-OCH_2Ph), 4.42 (dd, 1 H, $J_{H\text{-}2',\ H\text{-}3'}$ = 3.3 Hz, H-2′), 4.39 (dd, 1 H, $J_{H\text{-}2''',\ H\text{-}3'''}$ = 3.1 Hz, H-2‴), 4.36 (dd, 1 H, $J_{H\text{-}6''a,\ H\text{-}5''}$ = 4.6 Hz, $J_{H\text{-}6''a,\ H\text{-}6''b}$ = –10.2 Hz, H-6″a), 4.26 (dd, 1 H, $J_{H\text{-}6'a,\ H\text{-}5'}$ = 4.8 Hz, $J_{H\text{-}6'a,\ H\text{-}6'b}$ = –10.2 Hz, H-6′a), 4.18 (dd, 1 H, $J_{H\text{-}4''',\ H\text{-}5'''}$ = 9.2 Hz, $J_{H\text{-}4''',\ H\text{-}3'''}$ = 9.9 Hz, H-4‴), 4.17 (dd, 1 H, $J_{H\text{-}6'''a,\ H\text{-}5'''}$ = 4.8 Hz, $J_{H\text{-}6'''a,\ H\text{-}6'''b}$ = –10.4 Hz, H-6‴a), 4.10 (dd, 1 H, $J_{H\text{-}2,\ H\text{-}3}$ = 3.7 Hz, H-2), 4.07 (dd, 1 H, $J_{H\text{-}4'',\ H\text{-}5''}$ = 9.3 Hz, $J_{H\text{-}4'',\ H\text{-}3''}$ = 9.8 Hz, H-4″), 3.98 (dd, 1 H, $J_{H\text{-}6a,\ H\text{-}5}$ = 4.9 Hz, $J_{H\text{-}6a,\ H\text{-}6b}$ = –10.3 Hz, H-6a), 3.88 (dd, 1 H, $J_{H\text{-}4',\ H\text{-}5'}$ = 9.4 Hz, $J_{H\text{-}4',\ H\text{-}3'}$ = 9.9 Hz, H-4′), 3.85 (dd and dd, 2 H, $J_{H\text{-}6'''b,\ H\text{-}5'''}$ = 10.1 Hz, $J_{H\text{-}4,\ H\text{-}5}$ = 9.5 Hz, $J_{H\text{-}4,\ H\text{-}3}$ = 10.1 Hz, H-6‴b and H-4), 3.82 (ddd, 1 H, $J_{H\text{-}5,\ H\text{-}6b}$ = 10.3 Hz, H-5), 3.80 (dd, 1 H, H-3), 3.78 (dd, 1 H, $J_{H\text{-}6''b,\ H\text{-}5''}$ = 10.0 Hz, H-6″b), 3.73 (dd, 1 H, $J_{H\text{-}6'b,\ H\text{-}5'}$ = 10.0 Hz, H-6′b), 3.62 (dd, 1 H, H-3′), 3.55 (dd, 1 H, H-6b), 3.53 (dd, 1 H, H-3″), 3.52 (dd, 1 H, H-3‴), 3.40 (ddd, 1 H, H-5″), 3.31 (ddd, 1 H, H-5‴), 3.27 (ddd, 1 H, H-5′) ppm.

^{13}C NMR (150.9 MHz, $CDCl_3$, 25 °C): δ = 139.4 – 126.1 (arom. C), 103.3 (C-1‴), 101.9 (C-1″, 4,6-O*C*HPh, 4,6-O*C*′HPh), 101.5 (4,6-O*C*″HPh), 101.2 (4,6-O*C*′HPh), 100.5 (C-1′), 95.7 ($^2J_{C\text{-}1,\ P}$ = 4.9 Hz, C-1), 79.0 (C-3‴), 78.4 (C-4‴), 78.3 (C-4′), 71.2 (C-4, C-4″), 76.8 (C-3″), 76.2 (C-2″), 76.1 (C-3′), 75.9 (C-2‴), 74.9 ($^3J_{C\text{-}2,\ P}$ = 9.3 Hz, C-2), 74.6 (2‴-O*C*H$_2$Ph), 74.2 (C-2′), 73.4 (C-3), 72.2 (3‴-O*C*H$_2$Ph), 72.1 (3-O*C*H$_2$Ph), 71.3 (3′-O*C*H$_2$Ph), 70.8 (3″-O*C*H$_2$Ph), 69.9 [$^2J_{C,P}$ = 5.4 Hz, $^2J_{C,P}$ = 5.8 Hz, PO(O*C*$_a$H$_2$Ph), PO(O*C*$_b$H$_2$Ph)], 68.8 (C-6″), 68.7 (C-6‴), 68.6 (C-6′), 68.3 (C-6), 68.0 (C-5″), 67.7 (C-5′), 67.5 (C-5‴), 65.5 (C-5) ppm.

^{31}P NMR (242.9 MHz, $CDCl_3$, 25 °C): δ = –2.92 ppm.

HRMS: *m/z* calcd. for $C_{101}H_{101}O_{24}PNa$ $[M + Na]^+$: 1751.6318; Found 1751.6341.

β-D-Mannopyranosyl-(1→2)-β-D-mannopyranosyl-(1→2)-β-D-mannopyranosyl-(1→2)-α-D-mannopyranosylphosphate (18): Synthesized from **16** (25 mg, 0.014 mmol) according to the general procedure for hydrogenolysis of benzyl and benzylidene protecting groups. Filtration through celite with a 4:1 mixture of MeOH : H_2O yielded the product as a colorless oil. Yield: 7 mg (67%). $[\alpha]_D$ = – 15 ° (c = 0.01 H_2O, 24 °C). ^{1}H NMR (600.13 MHz, $CDCl_3$, 25 °C): δ = 5.47 (dd, 1 H, $J_{H\text{-}1,\ H\text{-}2}$ = 1.4 Hz, $J_{H\text{-}1,\ P}$ = 7.8 Hz, H-1), 4.96 (s, 1 H, H-1″), 4.94 (s, 1 H, H-1‴), 4.93 (s, 1 H, H-1′), 4.43 (d, 1 H, $J_{H\text{-}2'',\ H\text{-}3''}$ = 3.4 Hz, H-2″), 4.29 (d, 1 H, $J_{H\text{-}2',\ H\text{-}3'}$ = 3.4 Hz, H-2′), 4.16 (dd and d, 2 H, $J_{H\text{-}2,\ H\text{-}3}$ = 3.2 Hz, $J_{H\text{-}2''',\ H\text{-}3'''}$ = 3.4 Hz, H-2 and H-2‴), 4.00 (dd, 1 H, $J_{H\text{-}3,\ H\text{-}4}$ = 9.9 Hz, H-3), 3.94 (dd, 1 H, $J_{H\text{-}6'a,\ H\text{-}5'}$ = 3.1 Hz, $J_{H\text{-}6'a,\ H\text{-}6'b}$ = –12.3 Hz, H-6′a), 3.93 (dd, 1 H, $J_{H\text{-}6'''a,\ H\text{-}5'''}$ = 2.5 Hz, $J_{H\text{-}6'''a,\ H\text{-}6'''b}$ = –12.3 Hz, H-6‴a), 3.92 (dd, 1 H, $J_{H\text{-}6''a,\ H\text{-}5''}$ = 2.1 Hz, $J_{H\text{-}6''a,\ H\text{-}6''b}$ = –11.9 Hz, H-6″a), 3.87 (dd, 1 H, $J_{H\text{-}6a,\ H\text{-}5}$ = 2.0 Hz, $J_{H\text{-}6a,\ H\text{-}6b}$ = –12.3 Hz, H-6a), 3.84 (ddd, 1 H, $J_{H\text{-}5,\ H\text{-}6b}$ = 4.6 Hz, $J_{H\text{-}5,\ H\text{-}4}$ = 10.1 Hz, H-5), 3.77 (dd, 1 H, H-6b), 3.75 (dd, 1 H, $J_{H\text{-}6''b,\ H\text{-}5''}$ = 6.3 Hz, H-6″b), 3.73 (dd and dd, $J_{H\text{-}6'''b,\ H\text{-}5'''}$ = 6.5 Hz, $J_{H\text{-}3''',\ H\text{-}4'''}$ = 8.9 Hz, H-6‴b and H-3′), 3.72 (dd, 1 H, $J_{H\text{-}6'b,\ H\text{-}5'}$ = 5.5 Hz, H-6′b), 3.66 (dd, 1 H, $J_{H\text{-}3'',\ H\text{-}4''}$ = 10.0 Hz, H-3″), 3.62 (dd, 1 H, $J_{H\text{-}3''',\ H\text{-}4'''}$ = 9.5 Hz, H-3‴), 3.60 (dd 1 H, H-4), 3.58 (dd, 1 H, $J_{H\text{-}4'',\ H\text{-}5''}$ = 9.2 Hz, H-4″), 3.56 (dd, 1 H, $J_{H\text{-}4''',\ H\text{-}5'''}$ = 9.7 Hz, H-4‴), 3.51 (dd, 1 H, $J_{H\text{-}4',\ H\text{-}5'}$ = 10.2 Hz, H-4′), 3.42 (ddd, 1 H, H-5′), 3.39 (ddd, 1 H, H-5‴), 3.38 (ddd, 1 H, H-5″) ppm.

^{13}C NMR (150.9 MHz, $CDCl_3$, 25 °C): δ = 101.2 (C-1″), 101.0 (C-1‴), 99.1 (C-1′), 93.2 ($^2J_{C\text{-}1,\ P}$ = 5.8 Hz, C-1), 79.4 (C-2′), 78.9 ($^3J_{C\text{-}2,\ P}$ = 8.4 Hz, C-2), 78.4 (C-2″), 76.2, 76.2 (C-5″, C-5‴), 75.9 (C-5′), 72.9 (C-5, C-3‴), 72.1 (C-3″), 71.8 (C-3′), 70.4 (C-2‴), 68.9 (C-3), 67.3 (C-4), 67.1 (C-4′), 66.9, 66.7 (C-4″, C-4‴), 61.1 (C-6′), 60.7 (C-6‴), 60.5 (C-6″), 60.3 (C-6) ppm.

^{31}P NMR (242.9 MHz, $CDCl_3$, 25 °C): δ = 1.6 ppm.

HRMS: *m/z* calcd. for $C_{24}H_{42}O_{24}P$ $[M - H]^-$: 745.1804; Found 745.1835.

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